Developing Biosimilars and Biobetters

Parenteral Drug Association Metro Chapter
Somerset, NJ                   January 29, 2014

Michiel E. Ultee, Ph.D, CSO
Gallus Biopharmaceuticals, Princeton, NJ
Well-established CMO with decades of experience

- Mab’s
- Fusion proteins
- Other recombinant proteins
- All mammalian-cell culture

Full service
- Vial of Cells → Vials of Drug Product
- Process Development & Manufacturing
- Production, Purification, Filling
- Pure CMO -- Have none of our own products

Located in Princeton, NJ and St. Louis, MO

Attaching buffers to chromatography skid
What are Biosimilars & Biobetters?

► **Biosimilar**
► Recombinant protein therapeutics that resemble but are not identical to the original or reference product, *i.e.*, a generic biologic drug.
► Closely resembles reference product in safety, purity and potency
► Shows no clinically meaningful differences

► **Biobetter**
► Enhanced version of the reference product. Clinically meaningful differences are expected.
► Potentially an improved product in terms of efficacy, dosing, potency, etc. --- *i.e.*, a 2nd generation biologic drug
Why Biologics ≠ Standard Generic Drugs

- They are polymers of much larger size (100-5000X) and complexity than chemical drugs
- They are produced by living cells — It is almost impossible for the identical protein to be made by two different cell lines in different locations.
- All therapeutic proteins are a mix of closely related variants — Even run-to-run differences exist at a single manufacturer.

Figure from Shefali Kakar of Novartis, BioNJ Bio-breakfast Briefing on Biosimilars, June 2011
Why the Interest in Biosimilars?

► Follow the Money! Over $70B in biologics revenues open to competition from biosimilars in the next 5 years as patents expire

From Ronald Rader, *Bioprocess Intl* 11(6)s (June 2013)
How did Biosimilars Come About?

► Patient Protection and Affordable Care Act of March 23, 2010, aka “Obamacare”.

► Directed FDA to create an abbreviated pathway for biological products that are demonstrated to be “biosimilar” to or “interchangeable” with an FDA-licensed biologic.
Regulatory Progress

► Sets for key requirements for biosimilarity
► Primary sequence of protein (amino-acid sequence) must be identical
► “Totality of the Evidence” approach will be used by FDA, with comprehensive analytical analyses, animal and clinical data, to assess biosimilarity
► But, as of today (1/29/2014), no Biosimilars have received FDA approval

Guidance for Industry Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

DRAFT GUIDANCE
This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document contact (CDER) Sandra Benton at 301-796-2500.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

February 2012
Biosimilarity
Progress In Europe

- Well ahead of US. Initial Guidelines issued in 2005, 7 years before FDA
- Already 14 Biosimilars approved and on market, mostly smaller and simpler proteins.
- First more complex proteins, Monoclonal Antibodies (MAB), approved in Sept 10, 2013, with Biosimilar for Remicade (Infliximab) to Hospira & Celltrion.

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**PMLive**

First biosimilar mAb gets the nod in EU

Approval for Hospira's version of J&J's Remicade has global implications

Hospira and partner Celltrion have become the first companies to gain approval in the EU for a biosimilar monoclonal antibody (mAb), winning a green light for their infliximab-based products.

The biosimilar - which will be sold as Inflectra by Hospira and Remsima by Celltrion - will compete in the EU market with the original infliximab brand Remicade from Johnson & Johnson.

Remicade is approved to treat rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, psoriatic arthritis and psoriasis and last year racked up European sales of over $2bn.
Roadmap to a Biosimilar

- Collect Information
  - Public Information
  - Patents

- Market Surveillance
  - Target Product Profile

- Biosimilar Product Development
  - Cell Line Development
  - Process Development
  - Product Characterization
  - Analysis of critical attributes is conducted at all steps to ensure comparability with reference product

- CMC
  - Analytical Comparability

- Non-Clinical
  - PK/PD
  - Immunogenicity

- Clinical
  - Phase I: Clinical Equivalence
  - Phase III: In most sensitive indication
  - Pharmacovigilance

Adapted from Dr. Adriana Manzi, Atheln, Inc.
Target Product Profile

- Obtain multiple lots of Reference (Innovator) product and characterize with a set of state-of-the-art analytics, including bioassays.
- Define Critical Quality Attributes (CQA)
  - Physical, chemical, biological or microbiological properties that should be within a defined range to ensure the desired product quality.
  - Desired product quality = patient safety & efficacy
  - Refine ranges during program as product and process understanding grows
  - Set the “goal posts for each CQA by ranges found in innovator lots.
Analytical Analyses

- Side-by-side comparison to reference product in each assay is required to show comparability.
- Structural assays to assess structure and secondary modifications.
- Functional assays to assess target binding, cellular effects, selectivity and specificity.

Adapted from Dr. Emily Shacter, Chief, Laboratory of Biochemistry, US FDA, WCBP Biosimilar Strategy Forum, Jan 2012
Why is Glycosylation Important?

► Many complex proteins such as antibodies and enzymes are glycoproteins, containing from 2-30% carbohydrate.
► Glycosylation can affect a protein’s half-life (PK) and immunogenicity in the patient, as well as binding affinity, activity and stability.
► Glycosylation is complex
► Can be attached to protein either via Asparagine (N-linked) or Serine/Threonine (O-linked)
► Multiple sugar types, each with multiple attachment sites
Glycosylation Varies with Clone

CHOZN® GS⁻/⁻ IgG Producing Single Cell Clones
N-Glycan Clonal Variability – FB Process

% Relative Distribution

Slide courtesy of Dr. Kevin Kayser, SAFC
Glycosylation Varies with Basal and Feed Media

- Single Clone
- Single Mab
- Single Basal Media
  - A (top) or
  - B (bottom)
- Varied Feeds

Graphics courtesy of Dr. Kevin Kayser, SAFC
### Analytical Tools to Demonstrate Comparability

<table>
<thead>
<tr>
<th>Molecular Parameter</th>
<th>Attribute</th>
<th>Methods for control and characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary structure</td>
<td>Sum formula: Mass of light chain, heavy chain</td>
<td>LC-ESI-MS</td>
</tr>
<tr>
<td></td>
<td>Sum formula: Mass of intact MAb</td>
<td>LC-ESI-MS</td>
</tr>
<tr>
<td></td>
<td>Amino acid sequence</td>
<td>Orthogonal peptide maps with high resolution MS and MS/MS sequencing</td>
</tr>
<tr>
<td></td>
<td>Disulfide bridging</td>
<td>Non-reducing Peptide Map</td>
</tr>
<tr>
<td></td>
<td>Free cysteines</td>
<td>Ellman's, Peptide Map</td>
</tr>
<tr>
<td></td>
<td>Thioether bridging</td>
<td>Peptide map, SDS-PAGE, CGE</td>
</tr>
<tr>
<td>Higher order structure</td>
<td>Secondary and tertiary structure</td>
<td>CD spectroscopy, DSC, H-D-Exchange, FT-IR</td>
</tr>
<tr>
<td>Heterogeneity: C- and N-terminal</td>
<td>C-terminal: ±Lys, truncation to Pro-amide</td>
<td>CEX with/without CBP-digest; Papain-IEX; Peptide Map, IEF</td>
</tr>
<tr>
<td></td>
<td>N-terminal variants: (pGlu/Gln, pGlu/Glu)</td>
<td>CEX; Papain-IEX; RP-HPLC of LC, HC; Peptide Map, IEF</td>
</tr>
</tbody>
</table>

Adapted from Dr. Adriana Manzi, Atheln, Inc., April 19, 2012
## More Analytical Tools to Demonstrate Comparability

Adapted from Dr. Adriana Manzi, Atheln, Inc., April 19, 2012

<table>
<thead>
<tr>
<th>Molecular parameter</th>
<th>Attribute</th>
<th>Methods for control and characterization</th>
</tr>
</thead>
</table>
| Heterogeneity: Glycosylation | Glycan isoforms:  
• Major (G0, G1, G2)  
• Minor (e.g. Unfucosylated, α-gal) | NP-HPLC of 2AB-labeled glycans, coupled to ESI-MS, exoglycosidase digestion, MALDI TOF/TOF |
|                     | Sialic Acids incl. NGNA                 | NP-HPLC, WAX, HPAEC; RP-HPLC after DMB-labeling |
|                     | Aglycosylated MAb                       | CGE, Peptide map                          |
| Heterogeneity: Glycation | Glycation of Lys                         | Boronate affinity; LCMS; Peptide map      |
| Other amino acid modifications | Oxidation                              | RP-HPLC; Papain-HIC; Peptide map          |
|                     | Deamidation                             | CEX; Papain-LEX; Peptide map              |
| Heterogeneity: Size | Aggregation                             | SEC, FFF, MALLS, DLS, AUC; imaging methods and particle characterization |
|                     | Fragmentation at disulfides: HL, H\_L, H, L | CGE, SDS-PAGE, SEC, RP-HPLC               |
|                     | Fragmentation in amino acid chain: p100, p50 | CGE, SDS-PAGE, SEC, RP-HPLC               |

**Analytical chemist operating Multi-Angle Laser Light Scattering (MALLS)**
Cell-Line Development of a Biosimilar

► Genetics
► Use the same host cell or protein expression system as reference product, i.e., Don’t change the cell line.
► Base clone selection not only on productivity but also on product similarity to reference product

► Environmental
► Cell culture conditions, media and feeds affect both productivity and product quality.

► Analytical
► Your “eyes and ears” in this process to tell just how similar your protein is to the reference.
Immunogenicity: Ability to generate an immune response in the patient

Potential for any biologic

Loss of efficacy by binding up product and increasing clearance from the body

Adverse effects possible – anaphylaxis, immune cross reaction to patient’s own proteins

Biosimilar must have a comparable molecular profile regarding potentially immunogenic areas

Altered glycosylation

Aggregation

May develop changes leading to immunogenicity upon storage

IgG Antibody Structure Showing Potential Sites of Instability

(Diagram courtesy of Dr. Greg Kilby of Agilent Technologies)
Protein Purification for Biosimilars

► Route to removal of protein variants not found in purified reference product.
► Levels of impurities such as host-cell proteins (HCP) and DNA must be comparable to or lower than reference product.
► Yield is important to cost of goods.

Purification Scientist at chromatographic workstation
Protein Formulation

- Default is to use the same formulation as innovator
- But, new FDA Guidelines allow development of a biosimilar in a different formulation from reference product
- Potential advantage of delivery in a more convenient or stable form.
  - Self-Injection device like pen
  - Intranasal delivery (spray)
  - Transdermal (skin patch)
  - Lyophilized

Disposable Auto Injectors (Vibex™)
Pen Injectors
Reusable Needle-Free Injectors
Self-injection devices from Antares
Scale-Up and Production

- Comparable to new biopharmaceutical programs
- Side-by-side analytical comparison of your product to reference is part of entire process

2000L Bioreactor at Gallus Biopharmaceuticals
### Biosimilars vs. Biobetters

#### Table from Bill Stohl, Janssen, IBC Biopharm Prod Week, 29 Feb 2012, San Diego Slide 22

<table>
<thead>
<tr>
<th>Phase</th>
<th>Probability of Success (POS)</th>
<th>Novel NME</th>
<th>Biosimilar</th>
<th>Biobetter/Next Gen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical development</td>
<td>86% (industry average)</td>
<td>95%</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>Phase I clinical trials</td>
<td>84%</td>
<td>90%</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>Phase II clinical trials</td>
<td>53%</td>
<td>80%**</td>
<td>80%**</td>
<td></td>
</tr>
<tr>
<td>Phase III clinical trials</td>
<td>74%</td>
<td>--</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>Registration</td>
<td>96%</td>
<td>96%</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td>Total PTRS*</td>
<td>27% from entry into preclinical development</td>
<td>65%</td>
<td>41%</td>
<td></td>
</tr>
</tbody>
</table>

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# Using available data from multiple sources, including Reichert (2008), Paul et al. (2010), and DiMasi et al. (2010), [http://www.imgt.org/IMGTmedical/Overview_of_Drug_Development.pdf](http://www.imgt.org/IMGTmedical/Overview_of_Drug_Development.pdf), as well as other industry sources.

* PTRS, Probability of technical and regulatory success.

** Higher because target has been clinically validated.
Rational for Biobetters

- **Currency**: Why make a copy of a drug developed 15-20 years ago, when biopharmaceutical science has advanced so far since?
- **Analytics**: No need to slavishly match reference product with side-by-side analytics
- **Product Information**: Product indications and approximate dosages are known from reference product
- **Potential advantages**: Longer half-life, greater activity, lower dosage, and fewer side effects.
- **Cost of Goods**: Potentially lower if Biobetter is made using modern high-productivity methods and reference product is not.

Graphic from Dr. Susan Jones of Bioprocess Technology Consultants
Drawbacks to Biobetters

- Longer and more expensive clinical trials – must follow new-drug approval pathway
- How much better? Must be significantly better to gain acceptance over established reference product or biosimilars thereof.
- Large clinical trials likely needed to prove clinical superiority over reference product

Biobetter Program Strategy – How Superior?

- Biobetter research
  (Modified Fc, potency, half-life, etc.)
- Biobetter preclinical development
  (Safety and proof of improved pharmacology)
- Biobetter Phase I clinical development
  (Clinical safety, PK/PD, proof of improved pharmacology)
- Biobetter Phase II-III clinical development

Clinically Superior?
- Larger clinical trials to prove superiority for each indication

Superior Marketing Strategy?
- Trials powered to prove non-inferior efficacy but with improved pharmacology

Multiple indications?
- $1.2B question – can your Biobetter be best in class?

Graphic from Bill Stohl, Janssen, IBC Biopharm Prod Week, 29 Feb 2012, San Diego
Example of Biobetter of Protein C

- Protein C is produced in the liver and upon activation becomes a serine protease with multiple activities
  - Anti-coagulant
  - Anti-inflammatory
  - Cytoprotective
- Recombinant activated Protein C was sold for ten years until it was removed from the market in Oct 2011
- ZZ Biotech re-engineered the Protein C structure to reduce markedly the anticoagulant activity that was a major deterrent to use of Protein C in therapy of stroke and other neurological disorders.
- Gallus (as Laureate Biopharma) developed and produced this variant form for ZZ Biotech for Phase 1 trials, which were recently completed.

Graphic courtesy of ZZ Biotech
Conclusions & Recommendations

► Biosimilars allow broader patient access and lower-cost biopharmaceuticals
► Strong analytical techniques are essential to prove biosimilarity from the earliest stages of development
► Biobetters offer a route to an improved product but must be significantly better to be accepted.
► Recommend pre-IND meetings with FDA to review your strategy and development program

Biochemist developing purification process
For Additional Reading

- FDA Guidances for Industry, Quality & Scientific Considerations in Demonstrating Biosimilarity to a Reference Protein Product, [www.fda.gov](http://www.fda.gov)
- Elucidating Biosimilars Characterization, [Biopharm Int](https://www.biopharm-int.com), Sept 2013, 20-31
- Information on Gallus Biopharmaceuticals, LLC, [www.gallusbiopharma.com](http://www.gallusbiopharma.com)